Current Opinion in Colloid & Interface Science 28 (2017) 56–62

Contents lists available at ScienceDirect

Current Opinion in Colloid & Interface Science

journal homepage: www.elsevier.com/locate/cocis

Phase separation in food material design inspired by Nature
Or: What ice cream can learn from frogs

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A R T I C L E I N F O

Article history:
Received 28 February 2017
Received in revised form 12 March 2017
Accepted 13 March 2017
Available online 18 March 2017

Keywords:
Nature
Self-organization
Phase separation
Biopolymers
Cells
Structure
Dairy gels
Foams
Crystallization
Anti-freeze & ice-nucleating proteins

A B S T R A C T

Many foods are complex multiphasic systems prepared from raw materials and ingredients that are derived from complex hierarchically organized organisms such as plants, bacteria, yeasts or animals. The basic structural building blocks are biopolymers and colloids, which often maintain some of their original structure and functionalities even after extraction and refining. Of particular interest to food material science are the underlying physico-chemical mechanisms that are capable to drive and determine the organization, stability of structures and final functionality in both living and non-living systems. We give examples of phase separation phenomena due to thermodynamic incompatibility of biopolymers, complex coacervation and depletion interaction that play a role in both living organisms and foods. The mechanisms discussed are crucial to self-organization providing physiological benefits in living systems. We also present examples of ice crystal growth control in living systems at sub-zero temperatures. For both, phase separation and control thereof in the case of ice crystallization, we discuss how the corresponding structure-functionality relationship present in Nature can be used in the design of complex multiphasic food systems such as ice cream and dairy gels.

1. Introduction

Self-organization, based on complex hierarchical structures from the molecular level up to organelles, cells, organs and organisms, is a fundamental feature of biological systems and materials. Self-organization is a process by which a system, composed of several components with interaction rules, becomes organized in space and time. Self-organization leads to emergent properties, where the whole system exhibits characteristics that differ qualitatively from those of the single components without interactions [1]. The complex spatial arrangement of molecules is achieved through intermolecular forces that are determined by the structure of each molecule. The involved forces are of either short-range (e.g., adhesion and binding energies) or long-range 'colloidal' nature (e.g., leading to thermodynamic incompatibility) [2••,3••]. Self-organization is different from self-assembly as it relies on a continuous energy input to maintain the functionality. A constant energy supply strictly maintains the physico-chemical environment, such as temperature, electrolyte and pH balance, in a defined range. This defines the environment for molecular interactions and self-organization to occur, and allows living systems, contrary to most non-living materials, to be far away from a state of equilibrium. One might intuitively think that all bio-architectures are built by self-assembly; however, structure formation phenomena assisted by phase separation mechanisms between biopolymers appear to play a fundamental role in biological systems [4•,5••]. The concept of viewing the cytoplasm as a multiphase colloidal system of different protein phases was already postulated in the late 19th century by Wilson [6] who considered the cell to be densely packed with liquid 'coacervates'. Later on, Oparin [7] considered them an essential structural element in the primordial soup. However, these initial concepts of intracellular organization mediated through phase separation got no further attention as the focus shifted towards molecular biology. Nevertheless, phase separation phenomena have continued to attract considerable attention in non-living systems. In 1896, Beijerinck [8] first noted an 'incompatibility' in solutions of soluble agar with soluble starch or gelatine, which separated into two immiscible phases. Since then, significant understanding of the role of weak interaction forces in protein/polysaccharide mixtures has been created. The two main interactions between these macromolecules are either related to attractive forces leading to complex coacervation (association) or repulsive forces causing incompatibility (segregation) [9]. Mixtures of colloidal particles and (non-adsorbing) polymers may also phase separate due to the volume exclusion effect, resulting in a depletion interaction force which is controlled by

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http://dx.doi.org/10.1016/j.cocis.2017.03.002
1359-0294/

temperature, polymer size and concentration, respectively [10,11]. Recently biologists have renewed their interest in phase separation phenomena, studying intracellular organization mechanisms such as the formation of membrane-less organelles [4••,5••].

Nature has also evolved strategies that allow organisms to withstand adverse changes in external conditions by inhibiting irreversible structural damage. Frogs, insects and fishes that can survive freezing without the destructive effect of phase separation due to ice crystal formation and growth at sub-zero temperatures are fascinating examples of this. These organisms have specific proteins that influence the liquid–solid phase separation process upon ice crystal formation. Anti-freeze proteins influence the size of ice crystals by inhibiting their growth, whereas ice-nucleating proteins induce the nucleation process through the formation of embryonic ice crystals.

In our article, we will begin with an introduction to phase separation in Nature, more specifically in biology, and in food material design. We will then discuss examples of complex coacervation and depletion interaction in more detail, both in Nature and food material science applications. Finally, we will show how anti-freeze and ice-nucleating proteins are used to control the phase separation process of ice crystallization to preserve the integrity of cells and tissues at sub-zero temperatures, and in what way the same strategy can be used to improve the stability and quality of frozen desserts such as ice cream.

2. Phase separation for structure formation & self-organization

2.1. Phase separation in biology

Several studies provide compelling evidence that cell sub-compartmentalization is achieved by liquid–liquid phase separation [5••,12•,13••,14••,15,16]. This leads to the formation of 'membrane-less compartments' also called 'droplet organelles' exhibiting distinct phase boundaries. The local concentration of proteins and protein-associated molecules is increased in such organelles [17]. A number of cellular organelles such as the nucleolus, nuclear speckles, Cajal, P-, PML-bodies and stress granules among others have been shown to behave as membrane-less liquid droplets with mechanical properties that are different to their cellular environment [13••]. Many studies point to the importance of low-complexity proteins and RNA in determining the properties of phase separated droplet organelles. The absence of a lipid-based membrane to enclose the constituents of membrane-less organelles is advantageous in reacting to changes in the surrounding environment resulting in fast adaptation of their internal equilibrium. It has been proposed that release or sequestration of constituent proteins or RNAs from or within membrane-less organelles represents the mechanistic basis used in signaling cascades such as stress sensing [13••]. The transition from the mixed liquid state to phase separated liquid droplets and protein jamming or aggregation is tightly controlled by the cell. It has been indicated that deregulation during aging or due to genetic predisposition may result in pathological protein aggregation and thereby contribute to neurodegeneration and other protein related pathologies such as Parkinson and Alzheimer disease [12•,18•]. Understanding the involved mechanisms is of key importance for the development of new therapeutic approaches.

Coacervation in particular has been reported to also play an essential role in the formation of elastic fibers in the extracellular matrix of different vertebrate tissues such as blood vessels, lungs, skin, and elastic ligaments [19]. Coacervation-type mechanisms are also involved in the synthesis of the underwater bioadhesive of the Sandcastle worm, silk fibers of caddisfly larvae and plaques of mussels [20]. The insight gained in the mechanisms of biological adhesion phenomena is crucial to develop high performing underwater adhesives. Another example is the foam nest of the tungara frog that is stabilized by a dilute mixture of proteins and complex carbohydrates providing anti-microbial properties and anti-insect protection for the eggs [21••].

A new field of structural biology is therefore emerging, with the challenges to understand the subtle driving forces and structural bases of phase separation and protein aggregation phenomena and their role within intact cells as well as in *in vitro* systems. This will require the development of new biochemical tools and physical models to describe the panoply of weak interactions operating in these environments. Progress in this domain definitely requires a multidisciplinary approach involving competencies from cell biology, molecular and structural biology, mathematics and soft condensed matter physics.

2.2. Phase separation in food material design

Phase separation of polymers and colloids plays an important role in structure formation during processing and physical stability of the processed food. The phase separation threshold for biopolymer mixtures is usually below their concentrations found in food [22], a prerequisite for phase separation to occur. It should be stressed that unlike the other macromolecular compounds (the denatured globular proteins and polysaccharides) native globular proteins have an unusually high co-solubility. Some of them are probably co-soluble in all proportions in spite of great differences in amino acid composition. This feature is very important for the enzymatic function of proteins, both in Nature and food processing. As is well known in the Food Industry, thermo-mechanical treatments denature the globular proteins, decreasing their solubility and allowing them to contribute to the development of the final structure and texture through phase separation. Native proteins, denatured proteins and polysaccharides are essential food ingredients used to engineer desirable properties in food products and phase separation in food polymer mixtures. Together with applications for texture creation and modulation, these have been extensively reviewed in the past [22]. Macroscopic phase separation can be avoided by gelling the continuous phase, or both the continuous and dispersed phases.

The phase separation concept is also used to give food products functionalities beyond texture. One example is manufacturing of micro-hydrogel particles [23] that are typically biopolymer hydrogels in the form of microspheres, nanospheres (also called nanogels), spheroids and fibres. The utilisation of engineered microgels in foods has so far been limited, despite their great potential to address several needs in the Food Industry, for instance satiety control, encapsulation of phytonutrients and prebiotics, texture control for healthier food formulations (e.g., reduced fat products) and targeting delivery to specific areas in the digestive tract. Recently, McClements et al. described hydrogel strategies for creating reduced calorie foods, i.e., to mimic the properties of fat droplets and starch granules, i.e., particles that can vary considerably in size, shape, charge and behaviour [24]. The most commonly used building blocks for forming food grade microgels are proteins and dietary fibers. In some cases, other components such as minerals, acids, bases or enzymes are also used in the assembly process to help cross-link the biopolymer molecules together. However, the authors mention that there is still a way to go till the Food Industry will be able to use these structures to create reduced calorie foods with acceptable sensory properties. Protein/polysaccharide phase separation strategies are also used to create foods with improved sensory perception, such as mouthfeel, creaminess or taste and flavor perception [25•,26•] or for the microstructural design of foods for nutrition and health benefits [27,28]. Nicolai & Murray recently reviewed the effect of adding particles to phase separated mixtures of incompatible water soluble biopolymers. Besides the particle size, interactions of the particles with the biopolymers in the mixture and with each other at the interface appears to play a decisive role for stabilization [29••]. Improved understanding of particle stabilized water/water emulsions will also lead to innovative food systems with improved organoleptic and health properties.